REMARKS

In the Office Action dated August 3, 2005 (hereinafter, "the Office Action"), Claims 1-18, 20-25, 27, and 41-54 are under examination in this application. Claims 28-40 have been previously withdrawn.

Claim Rejections under 35 U.S.C. §103

A. The Examiner has rejected Claims 1-7, 10-12, 14-17, 19, 21-25, and 27 as being unpatentable over U.S. Patent No. 5,876,935 to Pankratz et al. in view of U.S. Patent No. 5,942,407 to Liotta et al. and further in view of U.S. Patent No. 4,091,277 to Doblhofer.

With respect to Claims 1, 2, 21, 23 and 25, the Examiner asserts that Pankratz et al. teach a method comprising the steps of combining with a sample a binding reagent labeled with a luminescent molecule that is capable of binding to an analyte, contacting the sample with another binding reagent that can be biotinylated, immobilized on a solid support such as superparamagnetic microspheres by means of avidin or streptavidin so that a complex with the analyte bound to the labeled binding reagent is formed, activating the luminescent label in the solid support-free sample or in the complex bound to the solid support, and determining the presence of analyte in the sample by detecting the light emitted from the activated luminescent label. *See* Office Action, page 3. The Examiner asserts that Pankratz et al. further teach that the label can be aequorin, activated by adding sufficient calcium ions. *See* Office Action, page 3.

The Examiner concedes that Pankratz et al. does not teach the presence of a calcium-caging compound in the support and does not teach the use of ultraviolet light to release calcium from the calcium-caging compound. *See* Office Action, page 3.

The Examiner relies on Liotta et al. as teaching the use of a calcium-caging compound immobilized in a support and the use of ultraviolet light to activate the compound. *See* Office Action, page 3.

On page 3 of the Office Action, the Examiner further states that Liotta et al. further teaches using a luminometer that is a compact photomultiplier for sensing the light. The Examiner concedes that Liotta et al. does not teach resetting of the photomultiplier after the pulse. *See* Office Action, page 3.

The Examiner relies on Doblhofer for the concept of resetting a photomultiplier. Specifically, the Examiner states that Doblhofer teaches a method wherein an output triggered by the trailing flank of a light pulse resets the integrator of a photomultiplier and further teaches that this provides for a photon detection and counting system which is accurate, but requires little in the way of apparatus and does not require expensive, highly accurate and rapid sophisticated electronic circuitry. *See* Office Action, page 3.

The Examiner uses these three references to find that it would have been obvious to include a caged calcium compound, immobilized in a support and ultraviolet light to activate the compound in the method of Pankratz et al. as suggested by Liotta et al. in order to extend the duration of light emission resulting from analyte detection. The Examiner asserts that it

would have further been obvious to reset the photomultiplier, as suggested by Doblhofer, in order to provide for a photon detection and counting system which is accurate, but requires little in the way of apparatus and does not require expensive, highly accurate and rapid sophisticated electronic circuitry. *See* Office Action, page 4.

Pankratz et al. discusses a chemiluminescent binding assay for measuring an analyte in whole blood without pre-treatment. The assay employs binding partners to the analyte, one of which is labelled with a luminescent molecule such as aequorin. The assay is carried out in solution, in a test tube or microtiter well. Pankratz et al., Column 5, lines 56 to 57. A calcium ion-containing solution is then added to the mixture to cause the aequorin to emit light, which is detected by a luminescence detector and correlates with the amount of analyte present.

It is Applicant's understanding that Liotta et al. discuss a method for activating light emission by a photoprotein by providing a substrate having a zone coated or impregnated with either a dried salt of a metal cation or a dried caged metal cation compound to produce a dried metal cation zone, allowing reagent to contact the dried metal cation zone and releasing metal cation from the dried metal cation zone to activate the photoprotein labeled reagent.

In describing the use of a calcium-sensitive photoprotein, it is indicated that when the soluble photoprotein encounters the reporter system zone of the described device, "calcium ions are locally released from an immobilized state", i.e. released from the dried state by hydration of the dried reporter zone containing a calcium salt. Liotta et al., Column 13, lines 25 to 28. This is the system used in the examples described in Liotta et al.

Liotta et al. then merely speculates that other techniques can be used to release calcium from the dried zone on hydration and proposes release of calcium from a caged calcium compound by introduction of ultraviolet light.

Liotta et al. do not consider any of the technical limitations or the problems to be overcome in supplying calcium in a caged form and releasing the calcium appropriately to activate a calcium sensitive chemiluminescent material.

If caged calcium is used as a calcium source, it is released by a pulse of ultraviolet light which can potentially interfere with the measurement of the light emitted from the chemiluminescent material.

Liotta et al. do not teach or suggest how to provide for an emission-free time period before reliable quantifiable light emission from the chemiluminscent material.

Previous work in the field of fluorescent measuring systems suggested the use of filters or shutters to separate light pulses, but this is a rather awkward and difficult solution. Further, in fluorescent systems, the light pulse provided to stimulate fluorescence is usually of much less power than the pulse required to release calcium from a calcium-caging compound at a level sufficient to activate emission of light from a chemiluminescent protein.

It was not appreciated until the work of the present inventors that, as discussed at page 15 of the specification as filed, the luminescent material could be selected, in combination with the caged calcium compound, to provide a "dead time" or period of no light between pulsing the caged calcium with ultraviolet light and the initiation of light emission by the

chemiluminescent as a calcium source in this type of chemiluminescence-based assay.

Doblhofer discusses a circuit to remove the effect of dark current in a photomultiplier tube. Dark current is external current that, under specified biasing conditions, flows in a photoconductive detector when there is no incident radiation. In effect, dark current is a false signal created within the photomultiplier tube. Doblhofer teaches a solution to a completely different problem– the problem of false signals, or artifacts, resulting from dark current and does not teach a manner of increasing accuracy with respect to secondary, unwanted, external light sources. For example, in the Abstract of Doblhofer, it is stated that switches are provided to close the circuit from the photomultiplier tube (PMT) to the integrator, connected with a photon emission event trigger circuit so that, under non event producing conditions, the integrator is disconnected from the PMT to prevent evaluating and possibly indicating spurious dark current noise pulses.

Since Doblhofer is directed to a method of artifacting and reducing noise produced from spurious dark current pulses, it is irrelevant to the invention at hand and would not be combined by one of ordinary skill in the art to arrive at the invention at hand. None of the three references cited by the Examiner discuss the problem solved by the present invention (that of needing to separate and only measure the light coming from the luminescent material and not the light used to activate the calcium-caging compound). Certainly, none of the presently cited references teach or suggest any method of solving that problem.

Applicant respectfully submits that there is nothing in the teachings of Liotta et al. which enable one of ordinary skill in the art to employ caged calcium compounds in an assay such as the assay described in Pankratz et al. and specifically no teachings regarding how to stimulate sufficient light emission from the chemiluminescent protein using a caged calcium compound without premature triggering of light emission or how to avoid interference between the ultraviolet pulse needed to break the caged compound and the emitted light which has to be measured. Liotta et al. provide nothing more than mere speculation and provide no motivation to employ caged calcium compounds in the method of Pankratz et al.

Applicant respectfully submits that Doblhofer is not relevant to the invention at hand and does not teach how to avoid interference between the ultraviolet pulse used to break the caged compound and the emitted light which has to be measured. Doblhofer discusses a method of avoiding inaccuracy in a PMT caused by spurious dark current noise pulses and is not relevant to separating or avoiding interference between two external light sources, namely, the ultraviolet pulse used to break the caged compound and the emitted light which has to be measured. Doblhofer does not teach any method of separating two external light sources, rather Doblhofer discusses a method of reducing internal noise within a PMTcircuit.

With respect to Claims 3 and 14, the Examiner asserts that Pankratz et al. teach that the method is an immunoassay for detecting and quantifying an antigen. *See* Office Action, page 4. Since Claims 3 and 14 are dependent directly or indirectly on amended Claim 1, it is respectfully submitted that these claims are patentable over Pankratz et al. at least per the patentability of Claim 1.

With respect to Claims 4, 5 and 6, the Examiner asserts that Liotta et al. teach the use of a calcium chelating agent such as EDTA or EGTA and that Pankratz et al. teaches that the solution may be whole blood. *See* Office Action, page 5. As Claims 4, 5 and 6 are dependent directly or indirectly on amended Claim 1, which as discussed above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claims 4, 5 and 6 are also patentable at least per the patentability of Claim 1.

With respect to Claims 7 and 17, the Examiner asserts that Pankratz et al. teach that the calcium-sensitive luminescent material is aequorin. *See* Office Action, page 5. As Claims 7 and 17 are dependent directly or indirectly on amended Claim 1, which as discussed above is patentable over Pankratz et al., it is respectfully submitted that Claims 7 and 17 are also patentable over Pankratz et al. at least per the patentability of Claim 1.

With respect to Claim 10, the Examiner asserts that Liotta et al. teach that the substrate can be nitrocellulose. *See* Office Action, page 5. As Claim 10 is dependent on amended Claim 1, which as discussed above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claim 10 is also patentable over these references at least per the patentability of Claim 1.

With respect to Claim 11, the Examiner asserts that Liotta et al. teach that the substrate comprises a transverse stripe with immobilized second binding partner and a calcium caging compound. *See* Office Action, page 5. As Claim 10 is dependent indirectly on amended Claim 1, which as discussed above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claim 11 is also patentable over these references at least per the patentability of Claim 1.

With respect to Claim 12, the Examiner asserts that Liotta et al. teach that the calcium caging compound is loaded with an excess of calcium, in order to overcome any residual chelating agents. *See* Offcie Action, page 5. As Claim 12 is dependent on amended Claim 1, which as discussed above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claim 11 is also patentable over these references at least per the patentability of Claim 1.

With respect to Claims 15 and 16, the Examiner asserts that Liotta et al. teach that the binding assay can be an immunoassay or a nucleic acid hybridization assay. *See* Office Action, page 5. As Claims 15 and 16 are dependent on amended Claim 1, which as discussed above is patentable over the cited references, it is respectfully submitted that Claims 15 and 16 are also patentable over these references at least per the patentability of Claim 1.

With respect to Claim 19, the Examiner asserts that Liotta et al. teach that the luminescence is measured by a photomultiplier. *See* Office Action, page 5. As Claim 19 is dependent on amended Claim 1, which as discussed above is patentable over the cited references, it is respectfully submitted that Claim 19 is also patentable over these references at least per the patentability of Claim 1.

With respect to Claims 22 and 24, the Examiner asserts Pankratz et al. teach that all the components may be added at the same time. *See* Office Action, page 5. As Claims 22 and 24 are dependent on amended Claims 21 and 23 respectively, and as amended Claims 21 and 23 are patentable over the cited references, as discussed above, it is respectfully submitted that Claims 22 and 24 are also patentable over these references at least per the

patentability of Claims 21 and 23, respectively. *See also* discussion below.

With respect to Claim 26, the Examiner asserts Liotta et al. teach that the timing of the caged calcium can extend the length of the light pulse and furthermore that Liotta et al. teach that the light detection is performed by utilizing a shutter assembly opened for a predetermined amount of time. *See* Office Action, pages 5-6. Claim 26 has been cancelled. It is, however, respectfully submitted that Liotta et al. do not teach time resolution of the UV pulse and the light emission from the chemiluminescent material as in amended Claims 1, 21 and 23, the light free time period being required to avoid premature emission from the chemiluminescent material interfering with reliable measurements. The shutter assembly referred to by Liotta et al. relates to an embodiment in which a photographic film or photographic plate is used in a combination with the described assay system. This shutter assembly is not relevant to the basic method of stimulating light emission by the chemiluminescent material by added calcium. As discussed in detail above, Liotta does not provide any teaching regarding how to avoid overlap between the UV pulse and the light emission by the chemiluminescent material or how to avoid premature triggering of light emission from the chemiluminescent material when using caged calcium compounds as a calcium source. It is respectfully submitted that the claims, as amended, are patentable over the cited references.

With respect to Claim 27, the Examiner asserts Liotta et al. teach the use of calcium chelating agents such as EDTA prior to the pulse of ultraviolet light. *See* Office Action, page 6. Liotta does not teach that the solution prior to the addition of free calcium should contain less than 20 nM of calcium. Indeed, as discussed more fully below, their suggested use of calcium at levels of 10 mM to about 100 mM would have been expected, if caged

calcium compounds were used, to produce a free calcium level considerably greater than 20 nM, as indicated in Ellis-Davies. Furthermore, if excess EDTA is used, then the released calcium would bind to the EDTA, as indicated by Ellis-Davies, leaving no calcium to initiate emission from the chemiluminescent material.

With respect to Claims 41-48, the Examiner has held that, where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art and that it would have been obvious through normal optimization techniques known in the art to load the calcium caging compound with up to 75% calcium and for the free calcium concentration of the solution to be less than 20 nM. *See* Office Action, page 6. General conditions of Claims 41-48 have not been disclosed in the cited references, as discussed above in relation to Pankratz et al., Liotta et al., and Doblhofer. Further, Liotta et al., teaches away from the present invention by defining calcium levels thought not achievable using caging compounds without triggering premature light emission. As such, the cited reference teaches away from loading the calcium caging compound with up to 75% calcium and for free calcium concentration of the solution to be less than 20 nM. More specifically, Liotta et al. stipulate that the amount of calcium in the reporter system zone of the described device is high enough to overcome the presence of the chelating agent present to prevent premature excitation of the photoprotein by calcium in the sample. Liotta et al. describe a typical amount of Ca^{2+} in the reporter zone as from about 10mM to about 100mM. This is consistent with other previously described assay systems such as Pankratz et al., who employed a calcium concentration of 100mM.

To deliver calcium at a concentration of 10mM to 100mM in the reporter zone using a caged-calcium compound, while maintaining a pre-release level of free Ca^{2+} of less than 20 nM, which is used to prevent premature photoprotein excitation, is generally not accepted as being technically possible. This is confirmed by U.S. Patent No. 5,446,186 to Ellis-Davies et al., cited by the Examiner, which shows, for example, that although the calcium-caging compound nitrophenyl-EGTA has a high affinity for calcium, and could be used locally to deliver a high concentration of calcium, the level of free calcium before the ultraviolet pulse would be much higher than 20 nM, thereby triggering premature light emission.

Although Liotta et al. make a vague suggestion that caged calcium compounds could be used, Liotta et al. do not address this anomaly. In fact, it was assumed in the art that caged calcium compounds could not be used as the calcium source in assays based on calcium-sensitive luminescent materials for precisely this reason, that the pre-release calcium level could not be kept low enough to avoid premature triggering of light emission from the luminescent material. It is not technically feasible to use a chelating agent such as EDTA to lower pre-release calcium levels while still being able to release enough calcium to trigger chemiluminescence, because the EDTA present at the time of calcium release would avidly take up released calcium.

Liotta et al., therefore, teaches away from the present invention by defining calcium levels thought not achievable using caging compounds without triggering premature light emission. As described in the present application, at page 14, a pre-release calcium level of than 20 nM is desirable. As seen in the examples, the calcium-caging compound is therefore preferably loaded to 75% or less with calcium.

Accordingly, Applicant respectfully submits that Claims 1-7, 10-17, 19, 21-27 and 41-48 are patentable over Pankratz et al. in view of Liotta et al., and further in view of Doblhofer.

B. The Examiner has further rejected Claims 8, 9, 13, 18, 20 and 49-54 under 35 U.S.C. §103(a) as being obvious over Pankratz et al. in view of Liotta et al., and further in view of U.S. Patent No. 5,446,186 to Ellis-Davies et al.

The Examiner asserts with respect to Claims 13 and 49-54 that Pankratz et al., Liotta et al. and Doblhofer teach a method of a binding assay as previously discussed involving use of caged calcium compounds but do not disclose specific caged calcium compounds. *See* Office Action, page 6. The Examiner further asserts that Ellis-Davies et al. teach a number of calcium-caging compounds and teach that these compounds produce very high yields of liberated calcium ion. *See* Office Action, page 7. The Examiner also asserts that it would therefore have been obvious to use DM-nitrophen or NP-EGTA as calcium-caging compounds in the method of Pankratz et al. and Liotta et al. *See* Office Action, page 7.

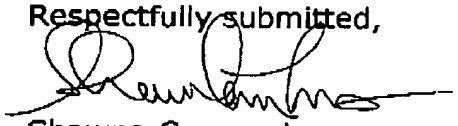
Firstly, as discussed more fully above, the discussions of Pankratz et al., Liotta et al., and Doblhofer cannot be combined to provide the method of amended Claims 1, 21 and 23. Furthermore, as also discussed fully above, the properties discussed in Ellis-Davies et al. for the calcium-caging compounds and the level of calcium described as being required by Liotta et al., ie. 10 mM to 100 mM, would lead one of ordinary skill in the art away from the claimed method, as one of ordinary skill in the art would assume that it was not possible to use such caged calcium compounds and avoid premature triggering of light emission by the chemiluminescent material.

Accordingly, it is respectfully submitted that Claim 13, which is dependent on amended Claim 1, is patentable over all of the cited references at least per the patentability of Claim 1.

With respect to Claims 8, 9, 18 and 20, the Examiner asserts that Liotta et al. teach the use of ultraviolet light in the form of a light pulse to activate the caged calcium compound, that Ellis-Davies et al. specify the use of a laser at 347 nm and that Liotta et al. further teach that a photomultiplier is used to sense the luminescence. *See* Office Action, page 7. As Claims 8, 9, 18 and 20 are dependent directly or indirectly on amended claim 1, which as argued above is not rendered obvious by Pankratz et al., Liotta et al. and Ellis-Davies et al., Claims 8, 9, 18 and 20 which are dependent directly or indirectly on amended Claim 1, are also non-obvious over all of the cited references at least per the patentability of Claim 1.

For at least the reasons set forth above, the Applicant believes that all of the pending rejections have been adequately addressed and that the present claims are in condition for allowance, which action is respectfully requested. The Examiner is invited and encouraged to contact the undersigned directly if such contact will expedite the prosecution of this application to issue.

Respectfully submitted,



Shawna Cannon Lemon
Registration No. 53,888

USPTO Customer No. 20792
Myers Bigel Sibley & Sajovec, P.A.
P. O. Box 37428, Raleigh, NC 27627
Telephone: (919) 854-1400
Facsimile: (919) 854-1401